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# Short report

## The *sac-4* gene of *Neisseria gonorrhoeae* and co-existing chlamydial infection

D J Phillips, C Patrizio, A Moyes, H Young

**Background/objectives:** Recently, the *sac-4* gene in *Neisseria gonorrhoeae* was postulated to increase the risk of developing mixed gonococcal and chlamydial infection. The aims of this study were to determine the frequency of the *sac-4* gene in a larger sample of isolates of different serovars and to assess the prevalence of *sac-4* in gonococcal isolates from patients with and without coexisting chlamydial infection.

**Methods:** Isolates from 259 episodes of gonorrhoea were tested by a PCR assay for the *sac-4* gene. The presence of co-existing chlamydial infection was determined from both laboratory and GUM clinical records.

**Results:** The overall prevalence of *sac-4* was 57.5% (149/259). The prevalence was not the same in all serovars and ranged from 34.9% in serovar 1B2 to 100% in serovar 1B18. Exact logistic regression analysis indicated significant differences in *sac-4* prevalence in isolates of different serovars. The prevalence of *sac-4* was 69.5% (41/59) in gonococcal isolates from patients with co-existing chlamydial infection compared with 57.9% (62/107) for those without chlamydial infection. Exact logistic regression analysis showed that the slightly increased *sac-4* prevalence among chlamydia positive patients ( $p=0.2$ ) virtually disappeared when serovar status was taken into account ( $p>0.9$ ).

**Conclusion:** The *sac-4* gene of the gonococcus does not increase the risk for mixed chlamydial infection.

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Keywords: dual infection; *Neisseria gonorrhoeae*; *Chlamydia trachomatis*; *sac-4*

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### Introduction

Dual infection of the genital tract with *Chlamydia trachomatis* and *Neisseria gonorrhoeae* is well established with a prevalence ranging from 14–43% but little is known of the epidemiology and associated clinical features of such co-existing infections.<sup>1</sup> Recently a gene, *sac-4*, was found in a high proportion of gonococcal isolates from patients with coexisting chlamydial infection and it was postulated that *sac-4* increased the risk of development of mixed gonococcal and chlamydial infection.<sup>2</sup> This gene conferred stable (not lost on subculture) complement factor C1q dependent serum resistance in gonococci.<sup>3,4</sup>

The aims of this study were to use the polymerase chain reaction (PCR) assay described by Nowicki *et al*<sup>2,3</sup> to determine the

frequency of the *sac-4* gene in a larger range of isolates of different serovars; and to assess the prevalence of *sac-4* in gonococcal isolates from patients with and without coexisting chlamydial infection.

### Materials and methods

#### PATIENTS AND ISOLATES

Isolates from 259 episodes of gonorrhoea in 80 female and 179 male patients attending a genitourinary medicine (GUM) clinic were studied. The isolates represented 19 different serovars. The prevalence of *sac-4* was determined for each of the common serovars, arbitrarily defined as comprising more than 15 isolates each: serovars represented by less than 15 isolates were grouped together as “other 1A” and “other 1B” serovars (see table 1). The presence of coexisting chlamydial infection was determined from laboratory and GUM clinical records.

#### EXTRACTION OF DNA AND PCR ASSAY

The growth from an overnight plate culture was mixed with 1 ml of saline, centrifuged, resuspended in 200 µl of distilled water, and heated for 20 minutes at 100°C. After cooling and centrifugation the supernatant from each tube was stored at –20°C for PCR testing.

Primers were as described by Nowicki *et al*<sup>4</sup>:  
Primer A, 5' TAT CTG CAG CAT CTC CTT TCC AAC C 3'  
Primer B, 5' TAG GAA TTC CTC TGA AGG TTA CGG 3'.

Table 1 *Sac-4* prevalence in various gonococcal serovars isolated from patients with and without coexisting chlamydial infection

Serovar	<i>Sac-4</i> * prevalence		
	Overall	Known chlamydia positive patients*	Known chlamydia negative patients*
1A2	80% (20/25)	69.2% (9/13)	100% (5/5)
Other 1A†	44.5% (8/18)	40% (2/5)	57% (4/7)
1B2	34.9% (22/63)	42.9% (3/7)	32.1% (9/28)
1B3	63.3% (19/30)	60% (6/10)	71.4% (10/14)
1B6	42.6% (20/47)	50% (1/2)	40.9% (9/22)
1B18	100% (16/16)	100% (7/7)	100% (8/8)
1B31	91.7% (33/36)	100% (8/8)	93.8% (15/16)
Other 1B‡	45.8% (11/24)	71.4% (5/7)	28.6% (2/7)
Total	57.5% (149/259)	69.5% (41/59)	57.9% (62/107)

\*Chlamydia status was not known for 93 patients.

†18 isolates representing serovars 1A1, 1A4, 1A5, 1A6, 1A8, and 1A21.

‡24 isolates representing serovars 1B1, 1B4, 1B7, 1B8, 1B13, 1B23, and 1B32.

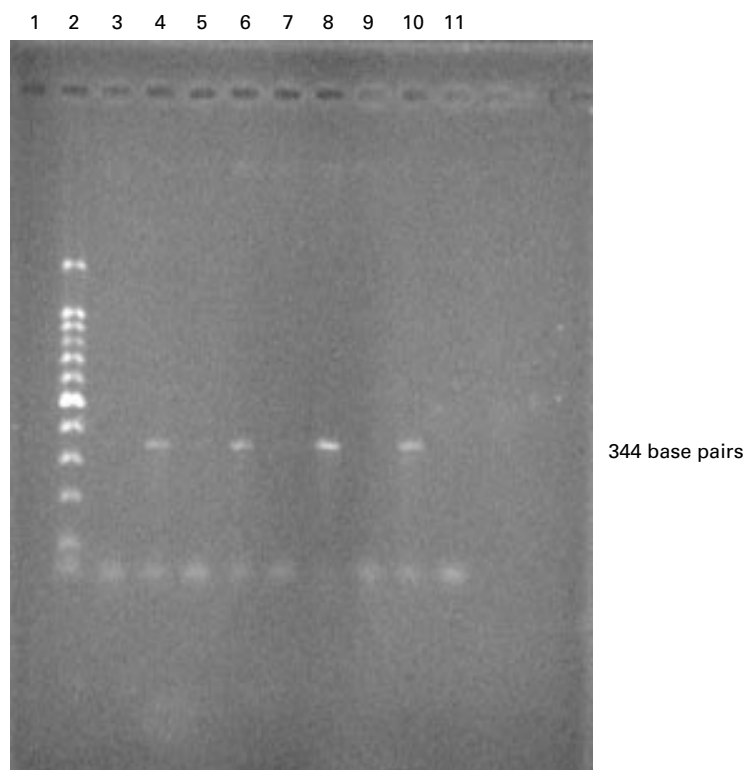


Figure 1 A typical gel showing products from the PCR assay for the *sac-4* gene of *Neisseria gonorrhoeae*. Lane 1, blank; lane 2, DNA ladder; lane 3, negative control strain 3701; lane 4, positive control strain 4520; lane 5, strain 1319 (*Sac-4*<sup>+</sup>); lane 6, strain 2925 (*Sac-4*<sup>+</sup>); lane 7, strain 1395 (*Sac-4*<sup>+</sup>); lane 8, strain 1023 (*Sac-4*<sup>+</sup>); lane 9, strain 3256 (*Sac-4*<sup>+</sup>); lane 10, strain 4098 (*Sac-4*<sup>+</sup>); lane 11, strain 2850 (*Sac-4*<sup>+</sup>).

A volume of 2 µl of DNA template was added to 100 µl reaction mix (50 mM KCl, 10 mM TRIS-HCl, 15 mM MgCl<sub>2</sub> and 0.1% Triton X-100) containing 0.5 units of Taq Polymerase (Promeg Corporation, Southampton), 20 µmol of each DNTP (Promega), and 0.1 µmol of each primer (Oswel DNA Service, Southampton). The reaction mixture was overlaid with two drops of liquid paraffin. The amplification reaction consisted of 30 cycles of 1 minute denaturation at 92°C, 1 minute annealing at 55°C, and 1 minute extension at 72°C. An aliquot of 20 µl was removed for electrophoretic analysis on a 2% agarose gel. A band corresponding to 344 base pairs indicated a positive *sac-4* result.

A *sac-4* negative and positive control strain were included in each run.

#### STATISTICAL ANALYSIS

This was performed using exact logistic regression.

#### Results

PCR analysis showed that 149 isolates (57.5%) were *sac-4*<sup>+</sup> (each strain gave a single band corresponding to 344 base pairs) and 110 isolates (42.5%) were *sac-4*<sup>-</sup> (no bands present). An example of a typical gel is shown in figure 1. The prevalence of *sac-4* ranged from 34.9% in serovar 1B2 to 100% in serovar 1B18 (table 1). The data in table 1 were analysed by exact logistic regression, with *sac-4* as the response (dependent) variable, and chlamydia status and gonococcal serovar as independent (ex-

planatory) factors with two and eight levels respectively. Comparing chlamydia positive with chlamydia negative patients unadjusted for serovar gave an odds ratio = 1.65, CI (0.8,3.47) *p*=0.2. This indicates that overall a higher prevalence of *sac-4* was observed in gonococcal isolates from chlamydia positive patients than in isolates from chlamydia negative patients but the difference was not statistically significant. Comparing the prevalence of *sac-4* in gonococcal isolates from chlamydia positive patients with chlamydia negative patients adjusted for gonococcal serovar gave an odds ratio = 1.06 CI (0.42,2.6) *p*>0.9. Thus the slight excess of *sac-4* prevalence in gonococcal isolates from chlamydia positive patients virtually disappears when the serovar of the infecting gonococcal isolate is taken into account.

Comparing the effects of gonococcal serovar adjusted for the chlamydia status of the patient, the odds ratios compared with serovar 1A2 were: other 1A serovars 0.19 CI (0.01–1.44); 1B2 0.14 CI (0.01–0.74); 1B3 0.44 CI (0.06–2.29); 1B6 0.11 CI (0.002–1.06); 1B18 undefined (this group had 100% prevalence for *sac-4*); 1B31 4.34 CI 0.32–251.66); other 1B 0.33 (0.05–1.84). These results indicate significant differences in *sac-4* prevalence between the serovar groups taking into account the chlamydia status of the patients. Exact logistic regression analysis for interaction between chlamydia status and gonococcal serovar yielded a *p* value of 0.05. Thus there is no evidence for differences between serovar groups in the comparison of *sac-4* prevalences between the chlamydia positive and chlamydia negative patients.

Similar results were obtained using approximate logistic regression including the subjects with unknown chlamydia status.

#### Discussion

The majority of isolates, 57.5% (149/259) in our local population of gonococci, were *sac-4* positive. However, the prevalence of *sac-4* was not significantly higher (*p*=0.2) in gonococcal isolates from patients with coexisting chlamydial infection (69.5%) than in those without (57.9%). These findings suggest that *sac-4* does not increase the risk of developing mixed infection with *C. trachomatis*. Our findings differ from those of Nowicki *et al*<sup>8</sup> who found *sac-4* in 71% (10/14) of gonococcal strains isolated from patients with mixed gonococcal and chlamydial infection and 7% (1/14) in isolates from patients with gonococcal infection only. Using exact logistic regression analysis we have shown that there are significant differences in the prevalence of *sac-4* between certain serovars.

Both epidemiological and biological factors may play a part in the occurrence of coexisting gonococcal and chlamydial infection. However our findings do not support a biological role for *sac-4* in the development of mixed infection.

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Mathematics and Statistics, University of Edinburgh for performing the statistical analysis.

*Contributors:* The study was designed by HY; CP set up the *sac-4* PCR assay in the laboratory; DJP performed *sac-4* testing of the isolates; AM performed serotyping and provided valuable comments in the development of the manuscript; DJP and HY were responsible for the analysis of the data and preparation of the manuscript.

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